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Role of Parasympathetic Nerves and Muscarinic Receptors in Allergy and Asthma

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Abstract

Parasympathetic nerves control the symptoms and inflammation of allergic diseases primarily by signaling through peripheral muscarinic receptors. Parasympathetic signaling targets classic effector tissues such as airway smooth muscle and secretory glands and mediates acute symptoms of allergic disease such as airway narrowing and increased mucus secretion. In addition, parasympathetic signaling modulates inflammatory cells and non-neuronal resident cell types such as fibroblasts and smooth muscle contributing to chronic allergic inflammation and tissue remodeling. Importantly, muscarinic antagonists are experiencing a rebirth for the treatment of asthma and may be useful for treating other allergic diseases.

The past century of research has identified common characteristics of allergic disease including increased smooth muscle contraction, hypersecretion, neuroplasticity and inflammation. The parasympathetic nervous system, a division of the autonomic nervous system, controls all of these events.

Although often unappreciated in the current literature, early studies highlight the importance of the parasympathetic nervous system in allergic disease by showing that surgical and pharmacologic denervation of parasympathetic nerves prevents disease establishment, disease progression, and symptom manifestation. Cutting nerve supply to the nose (vidian nervectomy) prevents allergy-associated overactive secretion [1] and cutting nerves supplying the airway (vagotomy) eliminated asthma symptoms and reduced inflammation [2]. Symptoms and allergic inflammation are also prevented by pharmacologic blockade of parasympathetic nerve signaling. Inhibition of parasympathetic nerves is accomplished by anticholinergic drugs, including ipratropoium and atropine, that block muscarinic receptors. Culminating decades of research, the first antagonist selective for a specific muscarinic receptor subtype, tiotropium, was recently proven to be clinically effective and was approved for treatment of poorly controlled asthma [3]. Here, we will discuss evidence that

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parasympathetic nerves represent an important common pathway for producing the symptoms of allergy and contributing to allergic disease progression.

Parasympathetic Signaling Controls Organ Functions Relevant to Allergy

Allergy is defined as a hypersensitivity reaction initiated by immunological mechanisms [3]. Allergens, or antigens that cause allergy, are typically foreign proteins that include pollen, pet dander, food, cockroach excrement, and fungal spores. The development of allergic disease in different organs often occurs together in the same person. Numerous studies have found positive associations between allergic rhinitis, asthma, atopic dermatitis, allergic conjunctivitis, and food allergies [4–12]. Irritable bowel syndrome (IBS) is also associated with allergy/atopy [9, 13–15]. Atopy is defined as the tendency, usually in childhood or adolescence, to develop sensitivity to common allergens [3]. The chronic-exposure allergies of the nose (rhinitis), airway (asthma), eye (conjunctivitis), gastrointestinal tract (food allergy, irritable bowel syndrome), and skin (atopic dermatitis, eczema) are characterized by acute and chronic symptoms including itch, pain, limited airflow, hypersecretion, intestinal dysmotility, and tissue remodeling. These manifestations of allergy are each wholly or partially under the control of the parasympathetic nervous system.

General Anatomy and Signaling of the Parasympathetic Nervous System

Parasympathetic nerves travel to end organs via a two-nerve pathway interrupted by the cell body of the second nerve. These cell bodies are ganglia and autonomic nerves are labeled anatomically as either pre- or postganglionic nerves depending on whether they supply the ganglia (preganglionic) or originate at the ganglia (post-ganglionic). It is the postganglionic nerves that release neurotransmitter onto end organs. Postganglionic nerves signal effector organs including submucosal glands, smooth muscle, and epithelial cells. The principle signaling mechanism between post-ganglionic parasympathetic nerves and effector target tissues is neuronal release of the neurotransmitter, acetylcholine, onto receptors sensitive to muscarine (muscarinic receptors) [16]. ACh activity is normally terminated by acetylcholinesterase [17].

It is worth noting that parasympathetic nerves also signal through nicotinic acetylcholine receptors and also release nonacetylcholine (noncholinergic) neurotransmitters such as vasoactive intestinal peptide (VIP) and nitric oxide (NO). Although VIP and NO contribute to vasodilation and mucus secretion in some organs, acetylcholine and muscarinic receptors typically provide dominant control of smooth muscle contraction and secretion caused by parasympathetic signaling.

Muscarinic receptors are also present on cells not innervated by the parasympathetic nerves including fibroblasts, and inflammatory cells [18, 19]. These cells may receive ACh that is released from non-neuronal sources such as epithelial cells [20].

Muscarinic receptors are a five-member family that belongs to the larger group of G protein-coupled receptors (GPCRs). For a more in depth review refer to muscarinic receptor classification by the International Union of Pharmacology [21]. The canonical intracellular signaling pathway of odd-numbered muscarinic receptors (M_1, M_3, M_5) is through G_q to

activate phospholipase C whereas the canonical signaling pathway of even-numbered receptors (M_2, M_4) is via G_i to inhibit adenylyl cyclase.

Parasympathetic Control of the Lung

Parasympathetic nerves supplying the lung, travel in the vagus nerve, maintain airway tone and play a prominent role in airway narrowing (bronchoconstriction) and in mucus secretion [22]. Action potentials generated in postganglionic nerves travel along short postganglionic fibers to smooth muscle and submucosal glands. Neuronal acetylcholine stimulates M_3 postjunctional muscarinic receptors on smooth muscle and mucus glands. Although M_2 receptors are also present on airway smooth muscle and M_1 receptors are on submucosal glands, cholinergic, M_3 receptor signaling is considered the principle mechanism for neuronally mediated bronchoconstriction and mucus secretion [22, 23]. This is supported in humans since the kinetically selective M_3 muscarinic antagonist, tiotropium, prevents methacholine-induced bronchoconstriction [24]. Non-neuronal epithelial release of acetylcholine stimulates airway epithelial ciliary transport via M_3 receptors to clear the airways but the contribution of parasympathetic nerves to this mechanism remains unknown [25]. M_2 receptors were also shown using knockout mice to inhibit acetylcholine- and M_3-mediated ciliary transport [25].

Inhibition of parasympathetic signaling is provided by M_2 muscarinic receptors on the parasympathetic nerves and by postjunctional VIP/NO signaling. M_2 receptors on nerves at the neuroeffector junction limit further acetylcholine release and thus provide negative feedback, limiting neurotransmission and bronchoconstriction [26, 27]. This was first demonstrated in guinea pigs by administering the selective M_2 receptor antagonist, gallamine, which blocked autoinhibition of acetylcholine release from parasympathetic nerves and in a dose dependent manner potentiated airway smooth muscle contraction induced by stimulating the vagus nerves [27]. The presence of inhibitory M_2 receptors on parasympathetic nerves supplying the lungs has been shown in all species studied so far including mice, guinea pigs, monkeys and humans [28, 29]. Parasympathetic nerves also release VIP and NO that can inhibit airway contraction and mucus secretion as shown in knockout mice, ex vivo airway tissue and release assays [30–34].

Muscarinic Receptor Changes on Airway Smooth Muscle

Whether changes in smooth muscle muscarinic receptor expression or function directly contribute to increased contractility in allergic diseases is controversial. Multiple studies found that airway smooth muscle isolated from asthmatics does not exhibit increased sensitivity to muscarinic agonists, suggesting that increased muscle contraction seen in vivo is not due to increased responsiveness to muscarinic signaling at the level of the smooth muscle [28, 35, 36]. In contrast, a few authors have shown airway smooth muscle from asthmatics exhibit increased contractile responses to direct stimuli in vitro presumably bypassing parasympathetic nerve signaling [37– 39]. The authors speculate that the differences between these studies and those that did not report a difference in asthmatic smooth muscle might be due to differences in the smooth muscle contractile agonist used (carbachol, ACh, histamine, adenosine) and the method of tissue harvest (bronchoscopic biopsy, postmortem harvest); however, a definitive study of muscarinic signaling that

includes selective muscarinic antagonists and direct muscle depolarizing agents (e.g. potassium chloride) have not been reported.

Animal studies of allergy have in some cases also reported increased airway smooth muscle contractility and mechanisms involving muscarinic signaling on the muscle. However, despite increased contractility to ACh, a rat model of allergic asthma showed no change in smooth muscle acetylcholinesterase activity, muscarinic receptor density or the ACh dissociation constant, and were unable to provide a physiological explanation of the increased contraction. However, there are low and high agonist affinity sites on muscarinic receptors and when studied in detail, ACh's affinity for the high-affinity muscarinic receptor-binding site was significantly greater in antigen challenged rats [40]. This selective increase in the high affinity ACh site on muscarinic receptors (presumably M_3) with no change in K_d could potentially increase G protein coupling which could increase bronchoconstriction, but this has not yet been linked to physiologic changes. An additional mechanism of increased ACh contraction was discovered in antigen-challenged dogs where isolated tracheal smooth muscle exhibited lower acetylcholinesterase activity than control animals [41]. Decreased acetylcholinesterase activity could increase acetylcholine levels seen by muscle and increase contraction. If inherent smooth muscle responsiveness is increasing it may also occur downstream of muscarinic receptors. This is supported by data that despite no change in muscarinic receptor density or K_d (shown above), airway smooth muscle from rats with airway hyperreactivity exhibit greater intracellular calcium levels (a surrogate measurement for myosin phosphorylation and smooth muscle contraction) in response to histamine [42]. Thus, while there is some evidence in vitro that contraction induced by muscarinic receptor agonists may be greater in muscle from asthmatic humans and antigen challenged animals, no clear mechanisms have been identified as yet, and in vivo, in animals, data supporting increased contraction is less compelling.

Dysfunctional Parasympathetic Nerve Control of Airway Smooth Muscle

Exposure of sensitized individuals to antigens increases contraction of airway smooth muscle mediated by parasympathetic nerves in humans and in animal models of disease [43]. In asthma, cutting the parasympathetic nerve supply to the airway (vagotomy) prevents increased smooth muscle contraction [2]. These findings in humans were replicated in animal models of disease. Multiple studies show that guinea pigs and mice that were sensitized to an antigen (ovalbumin or fungus) and subsequently exposed to that antigen, exhibit increased airway narrowing which is prevented by vagal section or by anticholinergic drugs [43–45]. In virally infected human asthmatics, anticholinergic treatment prevents increased bronchoconstriction and cough suggesting there is increased parasympathetic nerve activity in viral exacerbations of asthma [46, 47].

Studies have shown that autoinhibition of ACh release that is normally provided by inhibitory M_2 receptors on parasympathetic nerves, is disrupted in humans with asthma. Pilocarpine stimulates M_2 receptors and prevents reflex bronchoconstriction initiated by inhaled sulfur dioxide in healthy controls. However, pilocarpine has no protective effect in subjects with asthma, demonstrating that the neuronal M_2 receptors are not responding to an agonist [26]. Similar to humans, animal models of airway hyperreactivity exhibit

dysfunctional M_2 receptors in the airway. In control guinea pigs, blocking M_2 receptors with a selective antagonist potentiates vagally induced bronchoconstriction, but in guinea pig models of allergen-, virus-, or ozone-induced airway hyperreactivity, blocking M_2 receptors does not potentiate vagally induced bronchoconstriction demonstrating that neuronal M_2 muscarinic receptors no longer respond to ACh [18, 48–50]. Subsequent studies uncovered several mechanisms that result in loss of M_2 function including a role for inflammatory cells.

Eosinophils, seen commonly in asthma and allergy, produce and release cationic granule proteins including eosinophil major basic protein. Major basic protein is an allosteric antagonist for M_2 muscarinic receptors [51]. In animal models of allergic asthma, it appears that eosinophils are actively recruited to the lung and to airway nerves including parasympathetic ganglia [52] where they are activated. Disrupting eosinophil association with nerves or blocking eosinophil major basic protein prevents neuronal M_2 muscarinic dysfunction in vivo and prevents associated airway hyperreactivity in antigen-challenged, sensitized animals [50, 53, 54]. Thus, release of eosinophil major basic protein onto M_2 muscarinic receptors results in increased ACh and increased bronchoconstriction in allergic guinea pigs. New data show that blocking IL-5 with an antibody inhibits eosinophil recruitment to the lungs and prevents asthma exacerbations supporting that a similar ‘eosinophil-major basic protein-M_2 blockade-increased ACh release’ mechanism is present in humans with allergic asthma [55, 56].

Alternative mechanisms of M_2 dysfunction have been identified with other models of airway hyperreactivity. For example, viral neuraminidase decreases agonist affinity for M_2 receptors [57], and macrophages release interferon-gamma that decreases neuronal M_2 muscarinic receptor expression [49, 58]. While these have not been demonstrated in allergic asthma, these mechanisms may contribute to the heterogeneity of asthma, a disease difficult to subtype clinically without histologic analysis [59]. Despite asthma’s heterogeneity, in both humans and animal models most if not all disease response can be blocked by adequately inhibiting parasympathetic control with muscarinic antagonists [43].

Another intriguing mechanism of muscarinic antagonists is their ability to inhibit release of the inflammatory mediator, thromboxane A2 [60]. This is important because thromboxane A2 binds thromboxane receptors and potentiates the contraction caused by methacholine stimulation of M_3 receptors [60]. This is an additional mechanism whereby anticholinergic treatment could be beneficial.

Dysfunctional Parasympathetic Signaling and Airway Hypersecretion

Excessive mucus secretion in asthmatics contributes to airway obstruction and is also under parasympathetic control [22]. There is evidence of increased mucus glands (hyperplasia), increased mucus gland size (hypertrophy), and increased mucus secretion in asthma and allergy [59]. A link in humans between muscarinic receptors and mucus glands is shown in isolated airway tissue by administration of the muscarinic agonist, carbachol, which increases expression of mucin-related genes (e.g. MUC5AC) by PCR and protein by ELISA [61]. In two different studies, muscarinic antagonists, ipratropium and oxitropium bromide, inhibited the volume of airway mucus produced by patients with reversible airways

obstruction and chronic bronchitis [62]. It is important to note that the effect of muscarinic antagonists on mucus production remains controversial. Some studies report no change in mucus production after tiotropium treatment [63] but cite difficulty in separating mucus production from cough and mucociliary clearance [62]. However, in a mouse model of asthma, increased mucin gene production and decreased airway compliance (presumably due to increased mucus secretion) are prevented by a M_3 muscarinic receptor antagonist, aclidinium bromide [43]. Blocking M_3 with tiotropium in sensitized and antigen challenged guinea pigs also completely prevented the increase in mucus gland hypertrophy [64].

Parasympathetic Nerves and Muscarinic Signaling as Therapeutic Targets for Asthma

Historically, the widespread use of muscarinic antagonists has been tempered by problems including: under dosing, off target bioavailability, receptor subtype non-specificity, and short dissociation half-lives. In addition, beta-agonists and steroids have been beneficial in asthma [59, 65, 66]. Muscarinic antagonists have been re-introduced into therapeutic use as new and more selective drugs are developed coincident with a greater understanding of the role of muscarinic subtypes in disease.

Older studies demonstrated that nonselective muscarinic antagonists (e.g. atropine) cause significant reversal of airway narrowing in asthma [67]; however, they also produced unacceptable off-target side effects (e.g. dry mouth, tachycardia, confusion). Ipratropium, a newer nonselective anticholinergic drug, was developed to limit systemic bioavailability and decrease side effects but it did not significantly improve lung function in humans with COPD [68]. Importantly, although this lack of efficacy was probably caused by under-dosing ipratropium [18], the lack of effect was used for many years by the research community to discount the parasympathetic nerves contribution to asthma. Ipratropium's clinical dose had been determined using inhibition of bronchoconstriction induced by inhaled ACh rather than vagally released acetylcholine even though the later in humans represents physiologic control of airway smooth muscle. This is important because animal studies show that muscarinic antagonists in doses sufficient to block inhaled ACh-induced bronchoconstriction are not sufficient to block vagally-induced bronchoconstriction, demonstrating that ipratropium dosing is too low to block physiologic bronchoconstriction [69]. In patients with asthma, higher doses of ipratropium, 10-fold above current FDA recommended doses, significantly bronchodilated their airways [18, 70]. As a result, current expert opinion now recommends higher dose ipratropium for acute asthma exacerbations [66].

Under-dosing of cholinergic antagonists continues to be a major problem when evaluating studies of muscarinic antagonists, thus their therapeutic potential is still not fully realized. The newer generation muscarinic antagonists were developed to exhibit selectivity for M_3 over M_2 muscarinic receptors to minimize heart-related side effects [43], but additionally they also spare neuronal M_2 receptors. Tiotropium, a newer muscarinic antagonist with M_3 kinetic selectivity, was the first approved anti-cholinergic drug specifically shown to treat poorly controlled asthma. Remarkably, tiotropium was more effective at improving peak expiratory flow than steroids alone and was equivalent to combined steroid and long acting beta-agonist treatment [65]. Since dosing was determined similarly to ipratropium [18] this

suggests that tiotropium at higher doses than used in this study may further improve therapeutic efficacy [65]. Newer M_3 selective antagonists, bencycloquidium bromide and aclidinium bromide, have been tested in mouse and guinea pig models of allergic asthma and are equally, but not additively, effective as tiotropium. However, these new M_3 antagonists demonstrated faster onset of action, faster off rates (particularly at M_2), and rapid plasma hydrolysis important for limiting side effects [43–45, 71]. Aclidinium bromide is effective and well tolerated in COPD in a phase III trial [72], suggesting that it and other new antagonists could also be developed for treatment of asthma.

Parasympathetic Control of the Nose

The nose is supplied by parasympathetic nerves that control mucus secretion and nasal congestion due to edema, vasodilation, and sinusoidal engorgement [73]. Nasal preganglionic parasympathetic nerves travel in the petrosal and vidian nerves to synapse onto postganglionic nerves in the sphenopalatine ganglia. These postganglionic nerves project to the nasal cavity arteries, venous sinusoids, mucus-producing (seromucous) acinar glands, and goblet cells in nasal respiratory epithelium [73, 74]. Acetylcholine increases mucus secretion and nasal congestion and, as in the lungs, M_3 muscarinic receptors are the dominant subtype mediating secretion in the nose [26].

As in the lungs, M_2 receptor mRNA has been detected in human nasal mucosa and early pharmacologic data suggest M_2 receptors inhibit secretory reflexes [75]. Non-cholinergic parasympathetic neurotransmitters, VIP, PHM, and nitric oxide are also thought to cause nasal vasodilation and subsequent nasal congestion [73].

Dysfunctional Parasympathetic Signaling Causes Hypersecretion in the Nose

Hypersecretion (rhinorrhea) in allergic rhinitis is under neural control demonstrated most readily by a unilateral antigen challenge, to one side of the nose, causing a bilateral increase in secretion responses [76]. In humans with nasal allergy and in guinea pig models, studies report increased muscarinic receptors that correlate with hypersecretion following administration of muscarinic agonists, and in human's self-reported nose blowing frequency [1, 77, 78]. It is interesting to note that after sensitization to antigen, muscarinic receptor density decreases in nasal mucosa of guinea pigs [1]. However, this decrease in muscarinic receptors may be offset by increased agonist affinity as was shown in one study of nasal mucosa of humans with nasal allergy [77]. Increased agonist affinity was not however, found in an animal study [1]. Allergic rhinitis is also associated with increased activity of the enzyme responsible for acetylcholine synthesis, cholineacetyltransferase [1], so that ACh levels may be increased in nerves.

One potentially important mechanism of muscarinic receptor signaling in the nose is the hypertrophy and hyperplasia of mucus secretory cells. Repeated administration of muscarinic agonists (pilocarpine, methacholine) increased mucus cell hypertrophy and hyperplasia [30]. Muscarinic agonists may stimulate mucus cells via transactivation of epidermal growth factor that causes mucus cell and goblet cell activation and remodeling (hyperplasia and hypertrophy) [79].

Anticholinergic therapy for allergic rhinitis (discussed below) markedly reduced the daily duration and severity of rhinorrhea [80–82]. However, there are data that noncholinergic neurotransmission may also be important. For example, people with nasal allergies have more VIP-positive nerves in the nasal mucosa and also increased VIP release into nasal secretions [83, 84]. Increased VIP may contribute to increased nasal congestion in allergic rhinitis but this has not been directly tested.

Parasympathetic Dysregulation of Vasodilation and Vascular Permeability

In allergic rhinitis nasal congestion is not only produced by secretions but also by increased vasodilation that is under neural control. Administration of antigen or histamine to allergic patients on one side of the nasal mucosa led to closure of the opposite (contralateral) side of the nose demonstrating a neural reflex [76]. The muscarinic receptor antagonist, oxitropium, blocked histamine-induced nasal closure in allergic rhinitis patients demonstrating that muscarinic receptors are critical to this vasodilator response [85].

Parasympathetic Nerves and Muscarinic Receptor Signaling as Therapeutic Targets for Allergic Rhinitis

Allergic rhinitis is typically treated with antihistamines and sympathomimetics. However these have side effects including drowsiness and rebound congestion. Since the antihistamines, diphenhydramine and hydroxyzine, have beneficial off-target anticholinergic effects, specific muscarinic antagonists have been tested for rhinitis. In patients with perennial allergic rhinitis both the severity and duration of rhinorrhea was reduced after treatment with the anticholinergic drug, ipratropium bromide [81, 82], suggesting that anticholinergic drugs, especially the newer muscarinic receptor subtype selective drugs, may be added to the pharmacological options for rhinitis in the future.

Parasympathetic Control of the Eye

Parasympathetic control of the eye includes lens accommodation (focusing), pupil contraction, and production of the protective mucus layer of tear film [86–88]. Preganglionic parasympathetic nerves from the oculomotor nerve synapse with post-ganglionic nerves in the ciliary ganglion which project to eye muscles and conjunctival goblet cells [89]. Additionally, roughly 20% of cholinergic nerves innervating the conjunctiva project from a different parasympathetic ganglia called the pterygopalatine (sphenopalatine) ganglia. Accommodation, pupil contraction, and tear film production are thought to be mediated primarily by acetylcholine acting at M_3 muscarinic receptors. Tear production is increased by muscarinic agonists (carbachol, oxotremorine) and is blocked by M_3 antagonists both in vivo and in isolated lacrimal glands [90, 91]. Similarly, accommodation and pupil contraction both in vivo and in isolated ciliary muscle were stimulated by muscarinic agonists and inhibited by M_3 antagonists [92, 93].

Most noncholinergic parasympathetic innervation of the eye comes from the pterygopalatine ganglia (via the greater petrosal nerve), with a minority coming from the ciliary ganglion [94]. Noncholinergic nerves primarily release VIP and control intraocular pressure and blood flow [89, 95]. VIP has also been shown to stimulate mucus production and VIP

receptors are located on mucus-producing goblet cells [87]. In addition to VIP-containing nerves, the eye contains a dense network of parasympathetic nerves that release nitric oxide. These nerves have been demonstrated next to choroidal and scleral limbal blood vessels and presumably regulate blood supply [96].

Dysfuctional Parasympathetic Control of Lacrimation

Vernal keratoconjunctvitis is a seasonal eye allergy associated with atopy, elevated serum IgE, and increased tear production. Very little research has studied whether parasympathetic signaling or dysfunction may underlie increased tear production. However, one histologic study of nerves and muscarinic receptors has reported decreased M_1 receptors, decreased nerves, and disorganized expression of M_2/M_3 receptors (assessed by staining morphology) in conjunctival biopsies from vernal keratoconjunctivitis patients [97]. However, this is only one study and additional data are required. This same study reported increased VIP as another potential parasympathetic (but non-cholinergic) contributory mechanism to increased tear production [97]. The potential importance of VIP was also shown in one human trial where neuropeptides were quantified in tear film. Patients with allergic conjunctivitis had increased VIP in tear film as compared to healthy controls, but only after and not before conjunctival antigen provocation [98].

Parasympathetic Control of the Intestine

Intestinal parasympathetic nerves modulate peristalsis through control of the enteric nervous system [99]. Electrical stimulation of the vagal nerves or pelvic efferents innervating the stomach and intestine caused muscle contractions which were inhibited by muscarinic receptor antagonists and blockers of neuronal depolarization [100, 101]. Studies with selective muscarinic antagonists and muscarinic receptor knockout mice concluded M_3 muscarinic receptors are the dominant subtype mediating intestinal motility [102, 103]. In contrast, neuronal M_2 muscarinic receptors and noncholinergic neurotransmission typically inhibit intestinal motility [102, 103].

Parasympathetic Causes of Intestinal Dysmotility

Gastrointestinal allergy and irritable bowel syndrome (IBS), which are associated with allergy/atopy [13– 15], manifest in part through dysmotility of the intestine in humans as well as in animal models of the disease [13, 104, 105]. Parasympathetic dysfunction results in increased or decreased sensitivity to muscarinic agonists depending on the smooth muscle layer (inner circular vs. outer longitudinal), segment of the gastrointestinal tract, and disease model studied. The inner, circular, muscle layer of the jejunum and ileum in antigen-challenged mice and guinea pigs with ileitis are less sensitive to muscarinic agonist (carbachol)-induced contraction [106, 107]. In contrast, the outer longitudinal muscle layer exhibits hypersensitivity to carbachol in the same guinea pig ileitis model and also in a parasitic infection model [107, 108]. These data suggest that there may be a mismatch in activity of the inner and outer muscle layers that could lead to dysmotility. Furthermore, this could be mediated by the M_3 muscarinic receptor subtype. In a radiation-induced enteric inflammation model, intestinal dysmotility was prevented by chemical vagotomy of the small intestine using tetrodotoxin, blockade of ganglionic transmission with

hexamethonium, blockage of all muscarinic receptors with atropine, and blockade of M_3 muscarinic receptors on the muscle with the M_3 selective antagonist, 4-DAMP [109]. A change in muscarinic binding affinity, discussed above for asthma, represents a potential mechanism for increased muscarinic signaling in gastrointestinal dysmotility. In animal inflammation models, there was a selective increase in the high-affinity muscarinic binding site for carbachol which potentially increases smooth muscle contractility to acetylcholine [110].

Recent data has shown that crosstalk between eosinophils and parasympathetic nerves as discussed in asthma might also occur in gastrointestinal diseases [111, 112]. In intestinal biopsies from humans, mucosal eosinophils and eosinophil granule proteins are co-localized with nerves and myenteric ganglia. The authors speculate that eosinophils may contribute to intestinal dysmotility through a similar mechanism as seen in asthma, but this has not been directly tested [113].

Parasympathetic Nerves and Muscarinic Signaling as Therapeutic Targets for IBS

Several muscarinic receptor antagonists block intestinal dysmotility and provide relief with irritable bowel syndrome [114]. Muscarinic M_3 antagonists (zamifenacin, darifenacin) effectively inhibit intestinal motility and increase gastric emptying in diarrhea-predominant irritable bowel syndrome [114]. However, muscarinic antagonists alleviate pain which could confound mechanistic studies [114]. More selective muscarinic antagonists will help isolate the beneficial gastrointestinal motility effects versus nociceptive effects similar to treatment for chronic pain where M_4 agonists alleviate pain without eliciting cardiovascular side effects of M_2 stimulation [114].

Parasympathetic Control of the Skin

The skin is considered one of the three organs free from parasympathetic nerve control, however there is a small body of evidence showing direct parasympathetic control of facial blood vessels and sweat glands, and the presence of cholinergic, muscarinic, and VIP-ergic regulation of skin vasodilation and sweat secretion [115, 116]. Facial parasympathetic innervation was shown by tracing studies and immunostaining for vesicular choline acetylcholine transferase (marker for ACh synthesis), VIP, and acetylcholinesterase. Parasympathetic and sympathetic nerve fibers projecting to blood vessels in the lower lip are from the otic ganglion, a parasympathetic ganglion [116– 119]. Stimulation of the lingual nerve in the presence of a sympathetic nervous system blocker (guanethidine; that depletes neurotransmitters in the sympathetic nerves and thus isolates the effects of stimulation to parasympathetic nerves) caused dose-dependent increases in lower lip blood flow which were prevented by blocking ganglionic transmission with hexamethonium [118]. Although evidence for parasympathetic nervous function in the skin is limited to the lower lip, cholinergic nerves are capable of migrating into shallower layers of injured skin. Following sensory denervation, cholinergic (vesicular choline acetyltransferase positive) nerves were shown to migrate into the upper dermis past their normal depth and persist for at least 8 weeks [120, 121]. Whether these sprouting nerves are cholinergic sympathetic or parasympathetic nerves remain unknown.

In terms of atopic dermatitis which involves flexural skin locations (elbows, behind the knees) there is controversial data suggesting that cholinergic and noncholinergic signaling plays a role. In non-facial skin there are VIP-containing nerves and acetylcholinesterase-containing nerves and both have been shown to increase in nonlesional skin and decrease in lesional skin from patients with atopic dermatitis [122, 123]. However, other studies found no change in VIP levels in the skin from people with atopic dermatitis [124, 125]. Recent data has demonstrated that itch, the primary symptom of atopic dermatitis, probably involves M_3 muscarinic signaling in the skin. Itch induced by intradermal carbachol injection was prevented both atropine and 4-DAMP, an M_3 muscarinic antagonist [126]. A separate study showed a marked increased in ACh levels in the skin biopsies from atopic dermatitis patients, but choline acetyltransferase immunostaining was limited to non-neuronal cells (keratinocyptes, hair papilla, glands, endothelial cells, and mast cells) suggesting the increased ACh is due to increased non-neuronal ACh production [127].

Role of Parasympathetic Nerves in Inflammation and Disease Progression

Parasympathetic Proinflammatory Signaling, Recruitment, and Cell Adhesion

In allergic asthma increased parasympathetic nerve activity is correlated with airway inflammation and airway hyperreactivity [30, 43]. In addition, specific inflammatory cells are associated with nerves in allergic disease. In general muscarinic signaling is pro-inflammatory and results in production of inflammatory cytokines, chemokines, recruitment of inflammatory cells, and leukocyte adhesion. Therefore, muscarinic receptors and parasympathetic nerves may help establish allergic disease or may further disease progression.

In humans and animal models of asthma, eosinophils reside along nerves, inside nerve bundles, and adjacent to parasympathetic ganglia [128]. In a mouse model of allergic asthma, dendritic cells were also colocalized next to intrinsic (parasympathetic) ganglia and additionally airway sensory nerves [129]. Eosinophil recruitment to parasympathetic nerves in the airway involves cytokines (tumor necrosis factor), chemokines (eotaxin, MCP-3), neuropeptides (VIP, substance P, calcitonin gene-related peptide), and lipid mediators (leukotriene B4, platelet-activating factor) [130]. Eosinophil adherence to parasympathetic nerves is mediated by nerve expression of cell adhesion molecules that results in eosinophil activation and degranulation. Less is known about the recruitment of dendritic cells to parasympathetic nerves, but a recent study suggests nerves might activate dendritic cells. In a mouse model of allergic asthma, dendritic cell-nerve co-localization was associated with sites of T cell proliferation [131].

In studies of gastrointestinal disease and atopic dermatitis, eosinophils and mast cells are found near nerves; likely sensory nerves based upon the location and neurotransmitter content of the nerves. In a mouse model of eosinophilic gastrointestinal disease, electron microscopy demonstrated that eosinophils resided next to damaged small unmyelinated nerve axons [132] while in the skin of patients with atopic dermatitis, eosinophils and mast cells are increased and are in close proximity to nerves containing sensory-enriched neuropeptides [133, 134].

Inflammatory cells all express muscarinic receptors though the function of these receptors is not well studied. Mast cells contain M_1 [18], macrophages contain M_3/M_5 [18, 19], alveolar macrophages contain $M_1/M_2/M_3$ [19, 128], neutrophils contain M_4/M_5 [18] and eosinophils contain $M_3/M_4/M_5$ [18, 19]. In addition to the neuronal sources of ACh, many non-neuronal cells are now known to make and release ACh including epithelial and endothelial cells. For a more complete review, refer to Wessler and Kirkpatrick [20]. It is important to note that in allergic disease or following treatment with muscarinic antagonists, inflammatory cells can change muscarinic receptor subtype expression on their cell surface. For example, peripheral blood lymphocytes isolated from patients with asthma or allergic rhinitis had increased M_2/M_5 receptor expression. In contrast, M_3 receptors are not changed except in patients with severe allergy or with severe asthma in which case M_3 muscarinic receptors on inflammatory cells are decreased [135, 136]. In patients treated with tiotropium for 12 weeks sputum cells had responded to the M_3 antagonist by internalizing their M_3 receptors [137]. These studies suggest the response of inflammatory cells to neuronal or non-neuronal ACh may change in allergy and following treatment.

In some cells muscarinic receptors induce migration of inflammatory cells. For example, ACh causes macrophages to release a substance that is pro-migratory for neutrophils, an effect which is inhibited by M_3 receptor antagonists (4-DAMP, tiotropium) but not M_2 antagonists [18, 128]. Stimulation of macrophage muscarinic receptors with carbachol also increased migration of macrophages [19]. In obstructive airway diseases, muscarinic antagonists decrease eosinophil influx [43] which could indicate that muscarinic signaling mediates eosinophil migration. In one of the original studies of intractable asthma, surgically severing the vagus nerves resulted in a significant reduction of eosinophils in sputum and blood [2]. Mast cell recruitment is also associated with parasympathetic signaling. Cutting the parasympathetic supply to the nose significantly reduced mast cell density in the respiratory mucosa [138].

Depending on the inflammatory cell type and experimental setup, muscarinic receptor signaling either stimulates or inhibits release of pro-inflammatory mediators. Exogenous ACh stimulates peripheral blood monocytes to secrete leukotriene B4, a proinflammatory lipid mediator [139]. In alveolar macrophages, release of TNF-alpha, a potent inflammatory cytokine, and release of reactive oxygen species are stimulated by muscarinic agonists (carbachol) and are inhibited by the M_3 antagonist, tiotropium [128]. In contrast, muscarinic receptor signaling in mast cells may inhibit release of pro-inflammatory mediators although there is disagreement between in vivo and in vitro studies. Histamine release in isolated human airways, presumably by mast cells, is inhibited by exogenous ACh, a muscarinic agonist (oxotremorine), and by an acetylcholinesterase inhibitor (physostigmine). A role for muscarinic receptors was demonstrated since atropine reversed the ACh-induced inhibition of mast cell histamine release [140]. In contrast, an in vivo study of nasal allergy showed parasympathetic signaling might promote mast cell histamine release. When cutting the vidian nerve supplying parasympathetic nerves to the nose in patients with rhinitis, both the density of mast cells and histamine concentration in the nose were significantly reduced [138].

Muscarinic receptor signaling, through a host of different pathways, releases pro-inflammatory mediators from resident non-neuronal (noninflammatory) cells. For example, muscarinic receptor activation increases release of kinins, a family of pro-inflammatory mediators. In both humans with allergic rhinitis and antigen-challenged guinea pigs, methacholine administration leads to production of kinins, increased cytokine levels, and eosinophil infiltration [141, 142]. Ipratropium bromide inhibits this effect suggesting that muscarinic signaling indirectly increases cytokine (e.g. kinin) production and eosinophil influx [142]. For a complete review of the role of muscarinic receptors in airway inflammation, see Gosens et al. [30].

Muscarinic receptor signaling also stimulates proliferation and apoptosis of different inflammatory cells which potentially changes the overall inflammatory response. In isolated T and B cells, exogenous ACh promotes survival and stimulates proliferation [30]. This stimulatory effect of ACh may be through M_3 receptors and act differently on unique T cell subtypes. For example, blocking M_3 receptors on isolated human peripheral blood T cells with tiotropium reduced apoptosis of CD4+ but increased apoptosis of CD8+ T cells [143].

Parasympathetic signaling through nicotinic receptors also produces anti-inflammatory effects in humans with allergy and animal models [144]. In a mouse model of allergic asthma, a nicotinic agonist (1,1-dimethyl-4-phenylpiperazinium, DMPP), reduced inflammation and bronchoconstriction caused by the muscarinic agonist, methacholine [145]. However, exogenous nicotine administered to a rat model of allergic asthma decreases eosinophil influx and cytokine production but nicotine did not affect goblet cell metaplasia [146]. Thus, nicotinic signaling appears to be specific for inflammation and not for tissue remodeling or for mucus production. Nicotinic signaling might directly inhibit the influx of specific inflammatory cells since the nicotinic agonist, DMPP, has been shown to dramatically reduce chemotaxis and inflammatory cytokine release in isolated human eosinophils [147].

Involvement of Parasympathetic Nerves in Tissue Remodeling

Parasympathetic and muscarinic signaling has also been linked to underlying tissue remodeling seen in chronic allergic diseases. In general, muscarinic signaling promotes both the proliferation of smooth muscle and promotes phenotypic differentiation of mesenchymal cells and fibroblasts into a contractile phenotype.

In antigen-challenged guinea pigs, repeated challenge with antigen increased airway smooth muscle mass and smooth muscle myosin heavy chain expression in the main bronchi and this was replicated, ex vivo, in isolated smooth muscle [148]. In this study, the proliferative effect of muscarinic signaling likely acts through M_3 muscarinic receptors since tiotropium prevented antigen-induced airway smooth muscle proliferation [148]. Muscarinic receptor signaling also potentiated smooth muscle remodeling in human and bovine airways, an effect inhibited only by M_3 muscarinic receptor antagonists. As described in the nose, the proliferative effect of M_3 receptor signaling on smooth muscle is thought to involve epidermal growth factor and platelet-derived growth factor [30].

Stimulation of muscarinic receptors also increases fibroblast/mesenchymal proliferation [30] and importantly muscarinic receptors mediate the change of these non-contractile cells to acquire a contractile phenotype [30]. Inhalation of methacholine in humans in vivo causes myofibroblast proliferation and migration into the submucosa that occurs over a period of 2 weeks [149]. The mechanism of phenotypic transitioning by muscarinic signaling was shown to involve M_3 muscarinic receptors and various intracellular signaling molecules. In human fibroblasts the muscarinic agonist, carbachol, activates intracellular ERK1/2 and RhoA-GTP and upregulates collagen type I and a-smooth muscle actin consistent with transitioning to a myofibroblast contractile phenotype. These phenotype transitions caused by carbachol were dose-dependently inhibited by the M_3 antagonist, aclidinium bromide [150].

Indirectly M2-induced muscle contraction can increase muscle proliferation and switch non-muscle cells to a contractile phenotype. For example, muscle strain increases expression of smooth muscle actin and myosin heavy chain [30]. Since muscle strain is mediated by muscarinic receptor activation, this presents a physical rather than biochemical mechanism to increase muscle mass and contraction. Since different GPCR agonists, for example cysleukotrienes, also increase airway muscle remodeling, an effect that is prevented by a leukotriene antagonist, suggests that remodeling is a function of contraction and not activation of one specific GPCR [30].

Caveolin 3, a component of endocytosis, also represents an untested link between M_2 receptor signaling and muscle proliferation [151]. Cav3 knock-out mice show increased cardiac hypertrophy suggesting that caveolin 3 somehow prevents muscle proliferation [151] although this has not been tested in smooth muscle. Inhibition of caveolin 3 with methyl-beta-cyclodextrin inhibits M_2-mediated muscle contraction which could subsequently prevent contraction-induced muscle proliferation [152].

Muscarinic receptor signaling may also contribute to the barrier dysfunction of skin cells in atopic dermatitis by inducing early-in-life remodeling. Binding experiments and cAMP accumulation assays have shown muscarinic receptors are functional only in fetal skin fibroblasts and not adult fibroblasts [153]. Recent studies demonstrated that pilocarpine stimulates apoptosis in cultured neonatal skin fibroblasts through M_1 and M_3 muscarinic receptors [68].

Concluding Remarks

Parasympathetic nerves supply every major organ system associated with allergy and inflammation including muscle, glands and inflammatory cells. Thus, they are capable of inducing and modulating smooth muscle dysfunction or increased contraction, and increased mucus secretion. Importantly, the influence of the parasympathetic nerves extends to inflammatory cells which all express muscarinic receptors. Thus, parasympathetic nerves may modulate inflammation. Indeed, sectioning parasympathetic nerves decreases inflammation in the lungs and nose of asthmatic and allergic rhinitis patients respectively. Furthermore, parasympathetic nerves may also contribute to smooth muscle remodeling.

New therapies that target muscarinic receptors are in development for the treatment of COPD and asthma and they may also show promise in the treatment of allergic disease.

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